MARINE GENOME PROJECT

Pre-Project BioMap Survey Report

Tulum, Mexico (05/16/22-05/23/22)

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Introduction

At first glance it might look like underwater cave systems and the ocean have nothing In common. But as we continue to study these systems we are shocked by the similarities these two ecosystems have. Studying these environments can tell us a lot about the health of our ocean, and how we might better protect the life that calls the ocean home.

Underwater cave systems contain no light, and very little food/oxygen. Very little studies have been conducted on these unique environments. This is due to the complexity and difficulty getting into the underwater caves, among other barriers to studying underwater cave systems. (USGS, 2018)

Dissolved methane and other decaying organic materials, provide food for microbes and bacteria that larger species can feed on. Even the smallest of changes in ph, dissolved gasses, and salinity can cause irreversible damage to species living in and around the caves. (USGS, 2018)

By studying the species that call these harsh environments home we can better understand how to protect species in the depths of our oceans to the shallow shores of our coasts. Studying how species interact in the environment with little nutrients can help us understand how species might adapt to the changing conditions our ocean is facing. We can potentially use this information to help other species adapt and survive changing environmental conditions. The increased difficulty of surviving in underwater cave systems could reveal new proteins and enzymes that can be used for life saving medical purposes. The possibilities of increasing our scientific knowledge from studying underwater cave systems is immense.

In the Yucatan Peninsula it is estimated there are over 6,000 cenotes (Underwater Cave System, ceiling collapsed creating the opening) throughout the countryside. A majority of these systems have never been mapped or studied. The area is home to one of the longest underwater caves in the world that stretches for over 347 Kilometers or 215 miles. Within the Quintana Roo area there are many cenotes that have become popular with cave divers, swimmers, snorkelers and other outdoor enthusiasts. These cenotes provide income for some of the locals, some are used for drinking water and others are considered sacred sites by many natives. Thus it is imperative to our research team that we ensure the protection and honor the sacred beliefs of these amazing systems.

Almost yearly major new discoveries are being made in these unique environments. Some discoveries include discovering from ice age fossils, to human remains from hundreds/thousands of years ago, to new species that have never been discovered before. The possibilities for scientific discoveries are nearly endless.

One of the least studied and understood aspects of these systems is the life that calls these systems home. There are many isopod, crestaian and fish species that can be found in the cenotes. One of the limiting factors in studying these species is that using traditional methods we must visually find the species. These species are often incredibly small, translucent in body color and swim fast. This limits our ability to use traditional methods of monitoring species that would be performed in the ocean. By using new technologies we are able to study the biodiversity of these systems while limiting damage to ecosystems, the targeted species and the overall condition of the cenotes. The primary tool that will be used to study the species living in the cenotes will be eDNA (Environmental DNA).

All living things release cells into the local environment. Species shed hair, saliva, skin, blood, and bodily floods into the environment. By collecting the water, sediment and air from the environment we are able to extract the DNA from the species living in the immediate area. There are many benefits to eDNA over traditional direct issue DNA extractions. eDNA reduces the stress and injury on the targeted species, we can use it to identify multiple species in an area, and it is generally cheaper than alternative methods. (Thomsen & Willerslev, 2014)

Various environmental factors can affect how long DNA remains in the environment, such factors include but are not limited to; Ph, high temperatures, UV exposure, salinity, and chemicals. During our latest expedition to our survey sites we measured the Ph, temperature, and total alkalinity. By understanding these conditions we can better design our protocols to ensure ideal methods for ensuring optimal DNA extraction and storage.

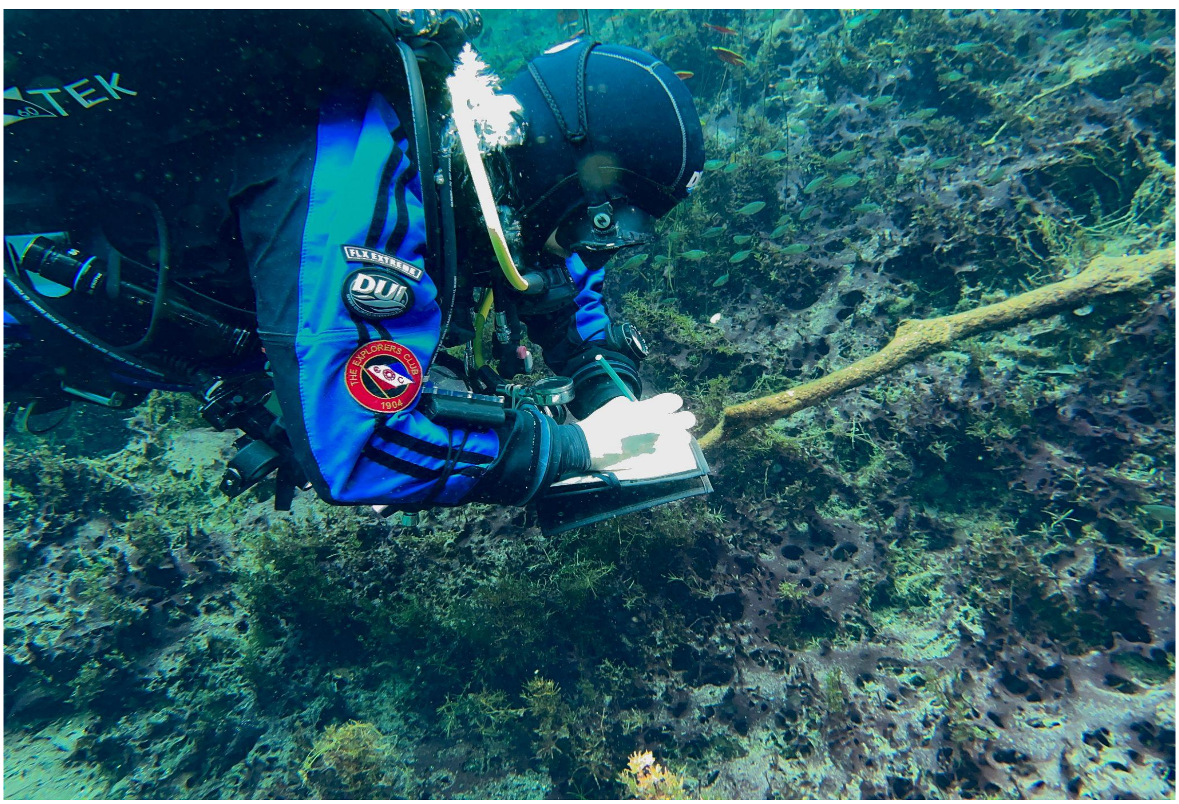
The MGP survey team collaborated with our counterparts at Third Dimension Diving to identify and locate cenotes to study. 3 Different cenotes were picked based on the following criteria; Changes to algae, plants/animals in recent months or years, Increase or decrease in visibility, tanic water, salinity within the cave, and finally any that have had drastic changes in human activity.

Later in the year the team will return to set up a permanent long term project to identify, monitor and track the changes to biodiversity in two cenotes.

Results

On the first day the MGP and 3D team went to cenote Carwash. 20.2742° N, 87.4862° W.

Site 1(Station 1) (In open water, Depth 12ft/3.6m, Temp 79f/26c) Location had active human influence by swimmers, snorkelers and other divers in the immediate area. Green algae covered most of the rocks in the area, along with other small vegetation. 5 different species of freshwater fish were present. What appeared to be a type of cyanobacteria was present on nearly 80% of rocks and vegetation, in some areas completely smothering vegetation underneath. The cyanobacteria can be seen in the photo below, red-maroon colored algae like material covering the vegetation in the bottom of the photo. Ph was recorded at 6.8, Total Alkalinity at 240ppm, and Cyanuric Acid 30ppm.



Site 1 (Station 2) (500ft/152m from station 1, Depth 24ft/7.3m, Temp 77f/25c) Station was located at the top of a steep slope, substrate underneath the collection location was mostly sand and crushed mineral from the ceiling. No life was seen in the immediate vicinity. Small amounts of dead organic material was present in the form of leaves, and bark. Location was about 150ft/45m away from another opening to the surface where human activity was present, and large amounts of fish, approximately more than 200 individuals. Ph was recorded at 6.2, Total Alkalinity at 240ppm, and Cyanuric Acid 0ppm.

Site 1 (Station 3) (1000ft/304m from station 1, Depth 24ft/7.3m, Temp 77f/25c) Location was at a slight decline. Mass amounts of organic materials we present on the floors, mainly vegetation. Blind fish and shrimp present in the immediate area, less than 10 individuals witnessed. Bottom substrate was made of sand, and broken mineral materials from the ceiling. Ph was recorded at 6.2, Total Alkalinity at 240ppm, and Cyanuric Acid at 0ppm.

On the second day the teams went to Maya Blue cenote 20.196999 N, 87.501018 W

3 stations were pre-selected for obtaining water samples.

Site 2 (Station 1) (In open water, Depth 9ft/3.6m, Temp 79f/26c) Location had green algae on a majority of rocky substrate. Small fish were present, only 2 different species were observed with less than 20 individuals in total. Location had active human activity from swimmers, and other divers in the immediate area. Ph was recorded at 6.8, Total Alkalinity at 240ppm, and Cyanuric Acid at 0ppm.

Site 2 (Station 2) (500ft/152m from station 1, Depth 45ft/13.7m, Temp 77f/25c) Location was above a mound of mineralized substrate. Some of the walls had what appeared to be gertite or similar dark mineral-like growth covering the upper walls. There was no salt intrusions present. No decaying matter was found. No life was found at the location. Ph was recorded at 6.8, Total Alkalinity at 240ppm, and Cyanuric Acid at 0ppm.

Site 2 (Station 3) (1000ft/304m from station 1, Depth 66ft/20.1m, Temp 77f/25c) Location was in the middle of a salt intrusion. Sample was collected from approximately 25ft above the floor. Sample was taken from in between the fresh and saltwater. There was no decaying material present. No life was present at the location. The floor was covered in gertite or similar dark mineral-like growth. Particulate was falling from the ceiling while the sample was being collected. Ph was recorded at 7.2, Total Alkalinity at 240ppm, Cyanuric Acid at 0ppm.

One the final survey day the teams went to Nohoch Nah Chich centoe 20.297221N, 87.404064 W

3 stations were pre-selected for obtaining water samples.

Site 3 (Station 1) (50ft into the cave within the light zone below an air pocket, Depth 9ft/2.4m, Temp 76f/24.4c) No algae growth was present. No decaying matter was found in the immediate vicinity. Small fish life was present, less than 30 individuals present. Human activity from swimmers in the immediate area. Floor was covered in sand and mineral particulates from the ceiling. Ph was recorded at 6.2, Total Alkalinity at 240ppm, Cyanuric Acid 0ppm.

Site 3 (Station 2) (500ft/152m from station 1, Depth 12ft/3.6m, Temp 76f/24.4c) Location had small amounts of decaying materials. Decaying material was mainly leaves and roots from trees in the air pocket directly above. Air pocket above has access to the open air but is not visible, nor accessible by humans. No human activity was present. Sample was collected in fresh water about 5ft/1.5m above the floor. The floor was made of minerals from adjacent walls and the collapsed ceiling. Ph was recorded at 6.2, Total Alkalinity at 240ppm, Cyanuric Acid 0ppm.

Site 3 (Station 3) (1000ft/304m from station 1, Depth 16ft/4.8m, Temp 76f/24.4c) Location had piles of decaying materials. Decaying materials was mainly roots and branches from tree roots in the ceiling above. No life was present in the immediate area. Air pocket above with light shining through. Direct access to the sun was through a hole about 10ft/10ft 3m/3m. No human activity was present. Sample was collected in the fresh water section about 5ft/1.5m above the floor. The floor was made of the same minerals as in the beginning stations. Ph was recorded at 6.2, Total Alkalinity at 240ppm, and Cyanuric Acid 0ppm.

Discussion

The results from the survey site allows our team to design our protocols to best optimize the preservation and successful extraction of the eDNA samples. The Ph results were interesting as there were sites that had a difference in ph readings at different station locations. Some of this variation could be due to human error, or proximity to human activity due to the increased acidity. Additional tests will be conducted during the project. The most surprising result was the Total Alkalinity readings. At all the sites we received total alkalinity results of 240ppm. Typical seawater is approximately 125ppm. Total alkalinity is a measurement of water's ability to resist changes to ph. Total Alkalinity indicates increased amounts of dissolved hydroxides and carbonates. It helps prevent the reduction in ph resulting in higher ph. The dissolved carbonates are most likely due to the area being mostly made of an old seabed composed of seashells and other carbonate based materials. Possible variability may be due to human error, testing sensitivity and/or contamination. Additional testing will be conducted during the project to test variation in total alkalinity based on wet/dry seasons.

Summary

Based on our pre-project survey, we will have to ensure that samples are protected during extraction and are stored immediately due to their exposure to more acidic conditions leading to faster DNA degradation. Our team will meet back with the 3D team to conduct training and finalize survey areas.. Preliminary results show that Maya Blue and Nohoch Nah Chich would be good sites for the long term project. This is due to their difference in geography, depth, abundance of life, and variation of salt intrusions.

Collaborators

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Resources

Thomsen, P., & Willerslev, E. (2014, December 18). Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. Retrieved May 26, 2022, from https://www.sciencedirect.com/science/article/pii/S0006320714004443

https://www.usgs.gov/programs/cmhrp/news/life-total-darkness-investigating-underwater-cave-ecosystems